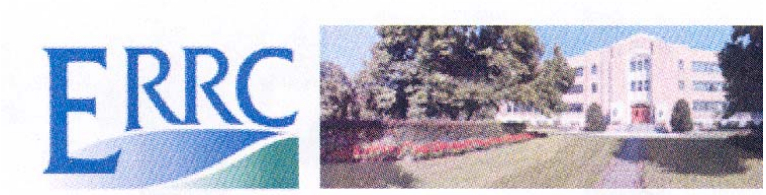

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Title: Description of Growth of *Clostridium Perfringens* in Cooked Beef with Multiple Linear Models

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Description of growth of *Clostridium perfringens* in cooked beef with multiple linear models

Lihan Huang*

The traditional linear model used in food microbiology employs three linear segments to describe the process of food spoilage and categorize a growth curve into three phases — lag, exponential, and stationary. The linear model is accurate only within certain portions of each phase of a growth process, and can underestimate or overestimate the transitional phases. While sigmoid functions (such as the Gompertz and logistic equations) can be used to fit the experimental growth data more accurately, they fail to indicate the physiological state of bacterial growth. The objective of this paper was to develop a new methodology to describe and categorize accurately the bacterial growth as a process using Clostridium perfringens *as a test organism. This methodology utilized five linear segments represented by five linear models to categorize a bacterial growth process into lag, first transitional, exponential, second transitional, and stationary phases. Growth curves described in this paper using multiple linear models were more accurate than the traditional three-segment linear models, and were statistically equivalent to the Gompertz models. With the growth rates of transitional phases set to 1/3 of the exponential phase, the durations of the lag, first transitional, exponential, and second transitional phases in a growth curve described by the new method were correlated linearly. Since this linear relationship was independent of temperature, a complete five-segment growth curve could be generated from the maximum growth rate and a known duration of the first four growth phases. Moreover, the lag phase duration defined by the new method was a linear function of the traditional lag phase duration calculated from the Gompertz equation. With this relationship, the two traditional parameters (lag phase and maximum growth rate) used in a three-segment linear model can be used to generate a more accurate five-segment linear growth curve without involving complicated mathematical calculations.*

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Introduction

Bacterial growth can be generally categorized into: lag, exponential, stationary, and death phases. Food microbiologists are mainly interested in the first three phases when considering food spoilage. Frequently, bacterial growth curves can be described by the lag time, maximum growth rate, and the logarithm of initial and maximum cell concentrations (Eqn(1)). Since most growth curves are sigmoidal in nature, the linear model may not depict the process of bacterial growth accurately. Other mathematical functions such as the

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Gompertz equation (Eqn (2)) are used frequently.

$$C = C_0, \qquad t \le L,$$
$$C = C_0 + k(t - L), \qquad L < t < A/k + L,$$
$$C = A, \qquad t \ge (A - C_0)/k + L, \qquad \text{Eqn (1)}$$

$$C = C_0 + B\exp\{-\exp[-\mu(t - M)]\}, \qquad \text{Eqn (2)}$$

where C is the logarithm of cell concentration, $\log_{10}$ cfu g^{-1}; C_0 the logarithm of initial cell concentration, $\log_{10}$ cfu g^{-1}; t the incubation time, h; k the exponential or maximum growth rate, $\log_{10}$ cfu g^{-1} h^{-1}; A the logarithm of the maximum cell concentration or the asymptotic cell concentration as t increases indefinitely, $\log_{10}$ cfu g^{-1}; B the maximum growth ($A-C_0$) or the asymptotic amount of growth as t increases indefinitely, $\log_{10}$ cfu g^{-1}; μ the relative growth rate of the Gompertz equation, h^{-1}; L the lag phase duration, h; and M the time at which point the absolute growth rate is the maximum, $\log_{10}$ cfu g^{-1} h^{-1}.

Mathematically, the maximum growth rate (k) is the tangent of the Gompertz growth curve at the inflexion point (M). It can be defined in Eqn (3) using two parameters (μ and B) of the Gompertz equation (Gibson et al. 1987, McClure et al. 1994). The lag phase duration (L) is the time at the intercept between the line tangential to the Gompertz curve at the time point M and the horizontal line $C = C_0$ (Eqn (4)). Graphically, the relationship between the linear model and the Gompertz equation is illustrated in Fig. 1.

$$k = \frac{\mu B}{e}, \qquad \text{Eqn (3)}$$

$$L = M - \frac{1}{\mu}. \qquad \text{Eqn (4)}$$

Although more accurate for describing the growth of micro-organisms as a function of time under isothermal conditions (Zwietering et al. 1990, 1994), the Gompertz model represents a better fit of the experimental growth data than the linear model. It offers no indication of the physiological states of the bacterial growth. The parameters used in the Gompertz equation lack biological basis (McDonald and Sun 1999). The linear model, on the other hand, is not adequate in describing the entire bacterial growth process. Generally, the linear model oversimplifies the bacterial growth process, which results in an underestimation at the transitional period between the lag and exponential phases and an overestimation between the exponential and stationary phases (Fig. 1).

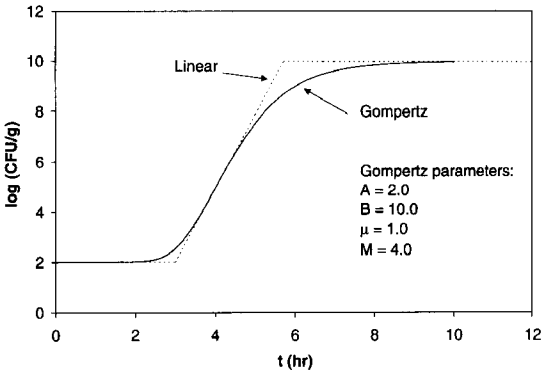

Figure 1. A typical microbial growth curve can be described by either a linear model or a Gompertz model.

The objective of this paper was to develop a methodology to mathematically categorize the different stages of bacterial growth and to improve the accuracy of the linear model in describing the growth process, particularly in the transitional periods between the lag and exponential phases and between the exponential and stationary phases. Such methodology will provide a clearer distinction of each phase and describe the bacterial growth more accurately.

Hypothetical growing stages

For any given moment in a population, the bacterial vitality is determined by the dynamic competition between growth and death (or negative growth). Depending on the stage of the bacterial growth, the rate of cellular multiplication and death changes with time. The net growth is the difference between the growth and death processes, and the overall rate of growth can be expressed as

$$k_t = k_g - k_d, \qquad \text{Eqn (5)}$$

where k_t is the overall rate of growth, $\log_{10}$ cfu g^{-1} h^{-1}; k_g the rate of positive growth, $\log_{10}$ cfu g^{-1} h^{-1}; and k_d the rate of negative growth (death), $\log_{10}$ cfu g^{-1} h^{-1}.

Within the lag phase, bacteria in the original inoculum adapt to a new environment. The bacterial cells generally increase in physical size, but not noticeably in number. If there are damaged cells in the initial inoculum, some of these cells may not survive the new environment and most likely die before the lag phase is completed. At this stage, k_g is 0, and k_d may be greater than 0. Depending on the amount of the damaged cells in the initial inoculum, a slight decline in the cell population can be observed frequently.

Near the end of the lag phase, some of the healthy cells begin to grow, and most of the damaged cells undergo repair. The number of bacterial cells in the population starts to accumulate. The rate of positive growth, k_g, becomes greater than 0 and the rate of death, k_d, remains 0. The bacterial growth is not synchronized. The overall growth rate, k_t, is slower than that of the exponential phase. This stage is the first transitional period or the phase of positive acceleration that prepares the cells for exponential growth.

After the first transitional period, the population begins to multiply synchronously at the maximum rate. This is the exponential growth phase. The bacterial cells undergo successive division cycles in a manner that doubles the cell numbers every generation time.

At the end of the exponential phase, the positive growth begins to de-accelerate, or the rate of negative growth (death) increases gradually. Each bacterial cell divides at a different rate and the overall growth rate (k_t) decreases gradually until a new equilibrium is reached. This stage is the second transitional period or the phase of negative acceleration.

At the end of this transitional period, a new equilibrium is established and the stationary phase starts. The positive growth rate (k_g) is equal to the negative death rate (k_d), and the cell concentration remains constant. The equilibrium will be maintained until the environment becomes hostile to the cells as a result of the accumulation of metabolic wastes.

Mathematical categorization of bacterial growth

The above hypothesis can be illustrated graphically in Fig. 2 with a hypothetical Gompertz growth curve (C_0=2, A=10, μ=1, and M=4). The entire growth process is divided into five linear segments: lag, first transitional, exponential, second transitional, and stationary phases with four intercepting points representing the end point of one stage and the starting point of a new stage. Two parallel linear segments (lines 0–1 and 2–3) are used to represent the transitional phases. These two linear segments are tangential to the Gompertz curve in the transitional regions at points Tr_1 and Tr_2.

With the two additional linear segments in the transitional phases, the lag phase ends at t_0, the intercept between the horizontal line (C=2) and line 0–1. The exponential growth curve (line 1–2) is the linear section tangential to the Gompertz curve at $t=M$, but starts at t_1, the intercept between lines 0–1 and 1–2, and ends at t_2, the intercept between lines 1–2 and 2–3. The stationary phase starts at the intercept between the second transitional segment (line 2–3) and the horizontal line C=10.

The slope (k_{Tr}) of the two transitional segments (lines 0–1 and 2–3) can be calculated from the first derivative of the Gompertz equation (Eqn (6)). Since there may be unlimited numbers of lines tangential to the transitional regions of the Gompertz curve, the transitional

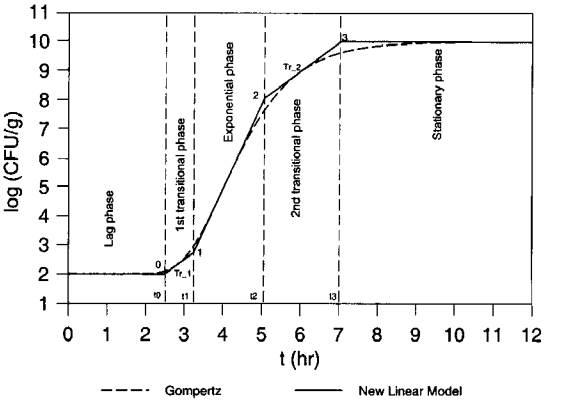

Figure 2. A microbial growth process can be divided into five different phases: lag, first transitional, exponential, second transitional, and stationary phases.

growth rate (k_{Tr}) can be assumed to be a fraction (α) of the maximum growth rate (k) in order to simplify the mathematical calculation. With a known k_{Tr}, two critical times (t_{Tr_1} and t_{Tr_2}) at the tangential points can be calculated from Eqn (6). The corresponding cell concentrations (C_{Tr_1} and C_{Tr_2}) can be determined from Eqn (2) after t_{Tr_1} and t_{Tr_2} are determined.

$$k_{Tr} = (A - C_0)\mu \exp \times \{-\exp[-\mu(t - M)]\} \times \exp\lfloor -\mu(t_{Tr\_i} - M)\rfloor, \quad i = 1 \text{ or } 2. \qquad \text{Eqn (6)}$$

After the slope of the transitional phases and the corresponding points (Tr_1 and Tr_2) tangential to the Gompertz curve are determined, linear equations representing lines 0–1 and 2–3 can be established (Eqn (7)). The intercepting points (0–3) between two sequential growth stages can be solved from a series of linear equations representing each growth stage. Consequently, the entire bacterial growth process can be described using a new set of linear equations (Eqn (8)). With this new linear equation system, the lag phase duration of bacterial growth is t_0, which is closer to the real initial value. The process represented by this set of linear equations can categorize clearly and describe more accurately the bacterial growth as a process.

$$C = C_{Cri} + k_{Tr}(t - t_{Tr\_i}), \qquad \text{Eqn (7)}$$

$$\begin{aligned} C &= C_0, \quad t < t_0, \\ C &= C_0 + k_{Tr}(t - t_0), \quad t_0 \le t < t_1 \\ C &= C_1 + k(t - t_1), \quad t_1 \le t < t_2, \\ C &= C_2 + k_{Tr}(t - t_2), \quad t_2 \le t < t_3, \\ C &= A, \quad t \ge t_3. \end{aligned} \qquad \text{Eqn (8)}$$

Materials and Methods

Organisms and inoculation of samples

Three strains of *Clostridium perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10288 (Hobbs serotype 13) were selected in this study. These strains were obtained from Dr John Novak of USDA-ARS-ERRC at Wyndmoor, Pennsylvania. Bacterial spores were ideal for this study since their growth covered the typical lag, exponential, and stationary phases categorized by traditional methods.

C. perfringens spores were prepared using the procedures developed by Juneja et al. (1993). Each spore crop was washed twice, resuspended in sterile distilled water, and maintained at 4°C until use. A cocktail was prepared by mixing aliquots of the spore suspensions having the same optical density. The spore cocktail was mixed into ground beef (93% lean) obtained from a local grocery store and previously irradiated to sterility by ionizing γ irradiation using a Cs^{137} source at the irradiation facility of USDA-ARS-ERRC (Thayer et al. 1995). The mixing was repeated in a Kitchen-Aid mixer (Model Max Watts 325) until a homogeneous distribution of bacterial cells was verified experimentally. The final concentration was approximately 100 spores per gram of ground beef. The inoculated ground beef (5 g) was packaged into plastic filter bags (Model BagPage® BP 100, Interscience Co.), vacuumed to remove air, and sealed at a final vacuum level of 15 mmHg. Samples were kept frozen (−20°C) until use.

Growth study

Frozen ground beef samples were thawed overnight in a refrigerator (≈4°C) and then heat-shocked at 75°C for 20 min to activate the spores and to inactivate any contaminating vegetative cells. The heat-shocked samples were rinsed briefly with running water (≈ 20°C for 1–2 min) and placed in incubators maintained isothermally at 17°C, 25°C, 30°C, 36°C, 45°C, 47°C, and 50°C, respectively. Samples from each incubation temperature were removed periodically for the determination of bacterial concentrations.

Determination of bacterial cell concentration

Samples removed from an incubator were diluted immediately with equal volumes (5 ml) of 0·1% sterile peptone-water. Since the beef protein was denatured during the heat-shock treatment, a rubber hammer was used to gently break and tenderize the meat samples. After

that, samples were mixed in a MiniMix Stomacher (Model BagMixer® 100 W, Interscience Co.) at the maximum speed for 12 min to homogenize the meat completely. After homogenization, a small volume (0·1–0·5 ml) of the liquid fraction was diluted serially with 0·1% sterile peptone-water and plated on Shahidi–Ferguson perfringens (SFP) agar. After spread plating, each SFP agar plate was overlaid with approximately 10 ml of freshly prepared SFP agar. Upon solidification of the overlay, the plates were placed in an anaerobic chamber (Model Bactron IV, Sheldon Manufacturing Inc., Cornelius, Oregon, USA) and incubated at 37°C for 24–48 h under an atmosphere of $CO_2/N_2/H_2$ (85%:10%:5%). Growth experiments for each temperature were replicated in triplicate to generate the isothermal growth curves.

Gompertz growth curves

The growth data from each experiment were fitted to the Gompertz equation (Eqn (2)) using NCSS 2000—a Windows-based statistical package (Hintze 1999). Four parameters of the Gompertz equation, C_0, B, μ, and M were determined using a nonlinear regression procedure provided in NCSS 2000. The lag phase duration and maximum growth rate of each growth curve were calculated using Eqns (3) and (4). Since nonlinear regression was used to obtain the Gompertz parameters, a pseudo-R^2 value was constructed for each Gompertz equation in NCSS 2000 to approximate the usual R^2 value used in linear regression. Although not a perfect indicator for the goodness of fit, the pseudo-R^2 value served well for comparative purposes.

Determination of multiple linear models

An analytical solution to Eqn (6) for t_{Tr_i} may not exist or may be very difficult to obtain since this equation is nonlinear. Therefore, a simple bisection method was used to solve this equation numerically (Norris 1981). To reduce the amount of numerical computation, the average growth rates in the transitional phases were considered the same. As a result, there were two solutions of t_{Tr_i} for each individual k_{Tr} in Eqn (6). To solve the equation numerically, the whole time domain was divided into two sections at $t = M$. One solution was found at $t \leq M$ and the other at $t > M$. For each Gompertz curve, a series of k_{Tr} values, ranging from 1/5 to 1/2 of the maximum growth rate k, was used to investigate the effect of k_{Tr} on the accuracy of the mathematical categorization of bacterial growth processes.

Effect of temperature on growth parameters

The effect of temperature on the relative growth rate (μ) of the Gompertz equation (Eqn (2)) was analysed using a modified Ratkowsky equation (Eqn (9)) to correlate μ and T (Zwietering et al. 1991). The modified Ratkowsky equation was also used to investigate the effect of temperature on growth parameters of the multiple linear models for a growth curve. The minimum and maximum growth temperatures determined from Eqn (9) were used to correlate the duration of each growing phase as a function of temperature (Eqn (10)).

$$\mu = A(T - T_{min})^2 \times \{1 - \exp[B(T - T_{max})]\}, \qquad \text{Eqn (9)}$$

$$\frac{1}{t} = a(T - T_{min})^2 \times \{1 - \exp[b(T - T_{max})]\}. \qquad \text{Eqn (10)}$$

Comparison of models

To compare the accuracy of different methods used to describe the bacterial growth process, cumulative relative error (CRE) defined in Eqn (11) was used. CRE is the summation of the absolute value of the relative error at each comparable data point. To compare the linear models with the Gompertz equation, equal numbers of data points for each temperature were generated to calculate CRE. To compare the linear models with the raw data, cell concentrations corresponding to each sampling time were calculated from the linear models and used to determine CRE for each growth curve.

$$\text{CRE} = \sum_{i=1}^{n} \left| \frac{C_i - C_{Li}}{C_i} \right|, \qquad \text{Eqn (11)}$$

where C_i is the cell concentration at t_i, raw data or calculated from the Gompertz model $\log_{10}$ cfu g^{-1} and C_{Li} the cell concentration calculated from the linear model, $\log_{10}$ cfu g^{-1}.

Statistical comparison

The general linear model (GLM) procedure of SAS Version 8.0 was used to compare the means of CRE for each growth model with the Tukey option used to group the means of each treatment.

Results and Discussion

The growth of *C. perfringens* in ground beef was sigmoid (Fig. 3) and could be fitted to the Gompertz growth model by nonlinear regression. The minimum pseudo-R^2 value was 0·985 among all the Gompertz growth curves. All nonlinear regression calculations converged quickly due to sufficient experimental data points generated for each growth curve. The relative growth rate (μ) of *C. perfringens* in ground beef was temperature-dependent, and could be expressed as a function of temperature using a modified Ratkowsky equation (Eqn (12)). This equation has a relatively high R^2 (0·912), and is plotted graphically in Fig. 4. Based on the modified Ratkowsky equation (Eqn (12)), the theoretical minimum and maximum growth temperatures for *C. perfringens* in ground beef were 10°C and 51·0°C in this growth study. The maximum growth temperature was validated experimentally in the laboratory as no growth was observed above this temperature. Both the minimum and maximum growth temperatures determined from Eqn (12) agreed closely with the values reported in the literature (Food and Drug Administration 1998, Juneja et al. 1999).

$$\mu = 7{\cdot}57 \times 10^{-4}(T - 10{\cdot}05)^2 \times \{1 - \exp[1{\cdot}007(T - 50{\cdot}82)]\}. \quad \text{Eqn (12)}$$

All of the growth curves could also be fitted with the traditional linear model (Fig. 5). De-

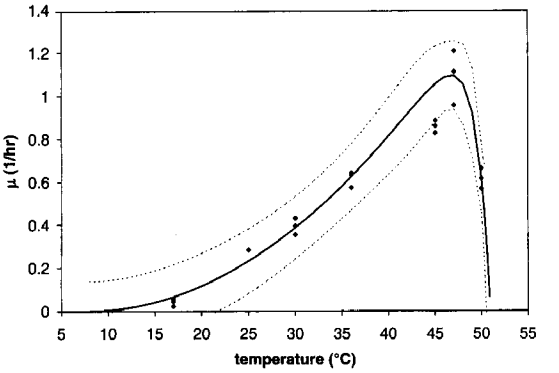

Figure 4. Temperature dependence of the Gompertz parameter μ can be described with a modified Ratkowsky equation. Dotted lines represent the upper and lower prediction limits at a 95% confidence level.

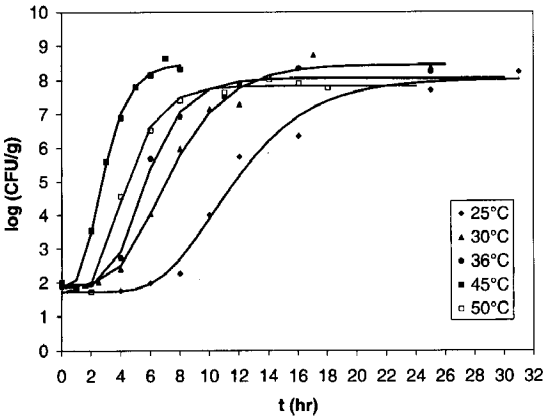

Figure 3. Sigmoidal nature of *C. perfringens* growth in ground beef at different temperatures. Solid lines represent growth curves fitted with the Gompertz model.

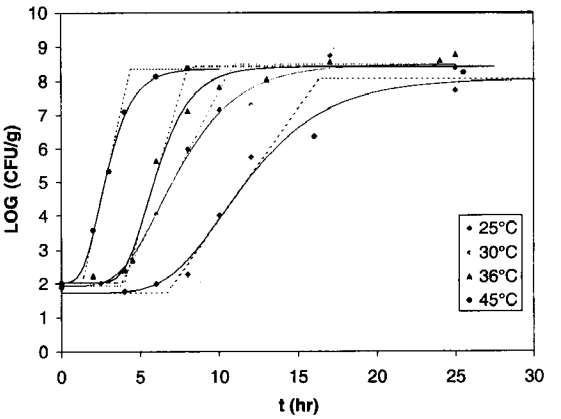

Figure 5. Traditional linear models can describe the growth of *C. perfringens* accurately only at certain portions of the growth curves.

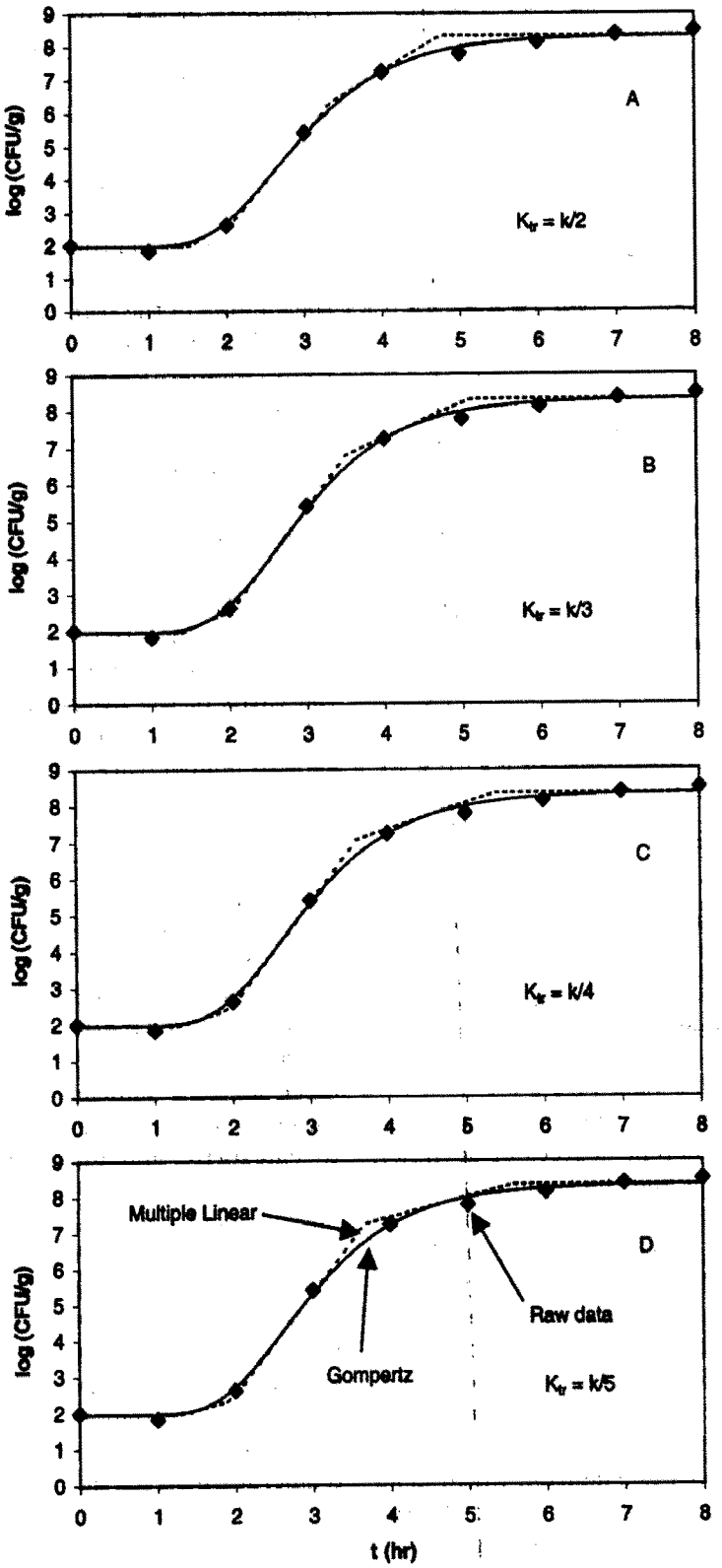

Figure 6. The accuracy of the linear method can be improved dramatically when multiple linear models are used to categorize and describe the bacterial growth process.

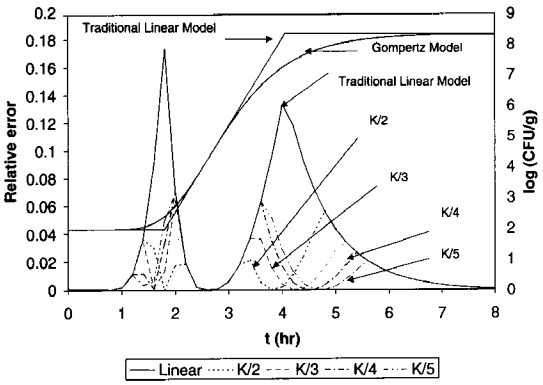

Figure 7. Comparison of error distributions in a growth curve fitted with different linear models.

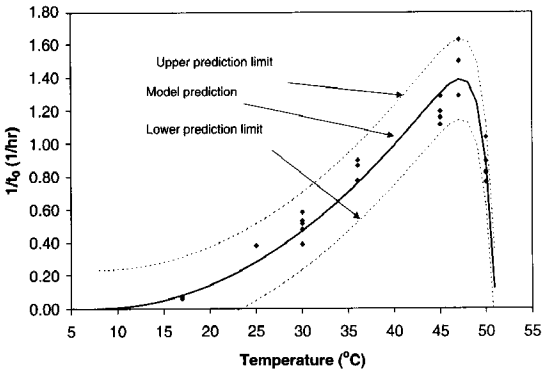

Figure 8. The duration of lag phase determined by the new method was a function of growth temperature. The inverse of the lag phase duration can be described by a modified Ratkowsky equation. Dotted lines represent the upper and lower prediction limits at a 95% confidence level.

pending on the nature of the sigmoidal curves, one can expect different degrees of deviation from both raw data and the Gompertz model. As illustrated in Fig. 5, the traditional linear growth model can best describe the growth of *C. perfringens* with high accuracy only at certain portions of the growth curves. Depending on the location in the growth curves, the absolute error calculated from the traditional linear model can be greater than 1 $\log_{10}$ cfu g^{-1}.

The accuracy of the linear model was improved dramatically when multiple linear models were used to categorize and describe the entire process of bacterial growth (Fig. 6). Graphically, the transitional linear segments, representing the unsynchronized growth in the transitional phases, were tangential to the Gompertz growth curve. Compared with the traditional three-segment linear growth curve, the five-segment linear model provided a much smoother transition between the two adjacent linear segments, particularly in the transitional regions. When relative errors, compared against the Gompertz model, are plotted against time on a growth curve (Fig. 7), the majority of the calculation errors are located in the vicinity of the transitional regions, and errors of the multiple linear models are substantially smaller than that of the traditional linear model. This observation agreed directly with the statistical analysis of the cumulative relative, or CRE, defined by Eqn (9). GLM analysis (Tukey grouping) on the means of CRE indicated that CREs of the traditional linear model for all the growth curves were significantly higher than those of the Gompertz model of the same growth temperature ($P<0{\cdot}05$). No significant difference was observed between the CREs of the five-segment multiple linear models and the Gompertz models of the same growth temperature, when compared directly against the raw growth data. This indicates that the five-segment multiple linear models are statistically equivalent to the Gompertz models. The four multiple linear growth models with transitional slopes ranging from 1/5 to 1/2 of the maximum growth rate for each growth curves were statistically identical ($P<0{\cdot}05$). This observation indicates that any one of these curves can be used to replace the traditional three-segment multiple linear curves. With these multiple linear models, it is now possible to categorize and differentiate accurately a growth curve in more detail, and to indicate physiological states of a bacterial culture.

In order to continue the analysis, the slope of the transitional regions was set as 1/3 of the maximum growth rate (k). Such selection was arbitrary since there was no difference in the multiple linear models with different slopes in the transitional regions of a growth curve. It is desirable to further investigate the effect of temperature on the duration of each phase (t_0, t_1, t_2, and t_3) in a growth curve.

The inverse of the lag phases (t_0) of the growth of *C. perfringens* in ground beef, determined from the multiple linear model with $k_{Tr} = k/3$, was correlated to the temperature using

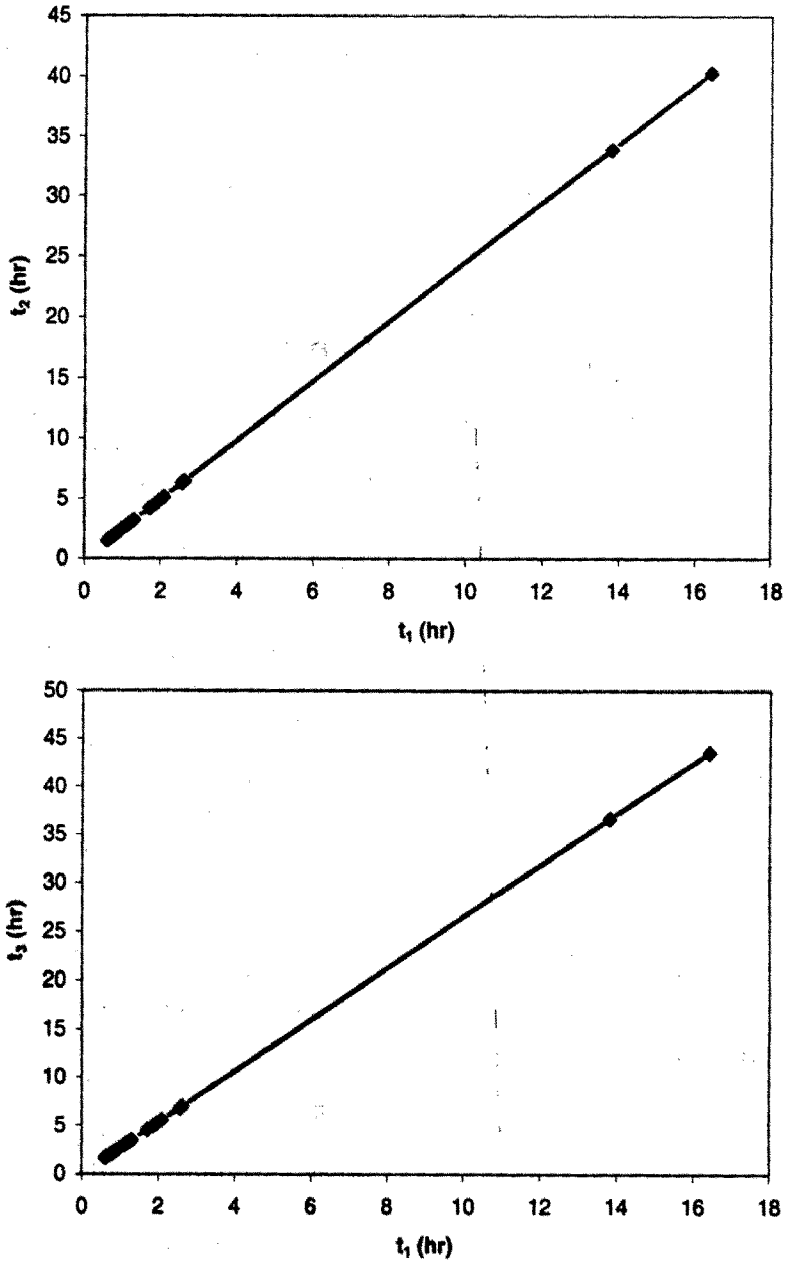

Figure 9. The duration of each growth phase after the lag period is directly proportional to each other.

a modified Ratkowsky equation (Eqn (13)). This equation has a pseudo-R^2 value of 0·922, and can describe adequately the relationship between $1/t_0$ and temperature (Fig. 8).

$$\frac{1}{t_0} = 1{\cdot}083 \times 10^{-3}(T - 10{\cdot}05)^2 \times \{1 - \exp[0{\cdot}859(T - 50.82)]\}. \quad \text{Eqn (13)}$$

The duration of each phase after the lag period was directly proportional to each other (Fig. 9). The durations of the exponential and the second transitional phases could be expressed as a function of the duration of the first transitional phase (Eqn (14)). The R^2 for both models was 1·0 using the linear regression. The interdependence of t_1, t_2, and t_3 was

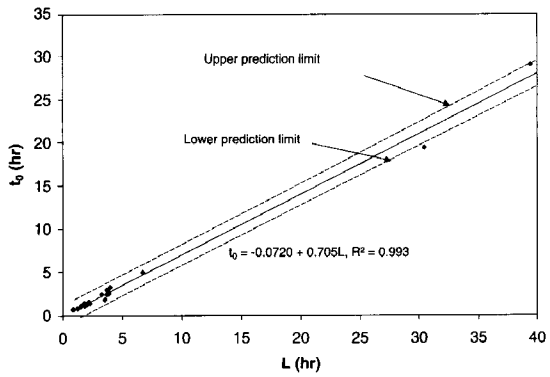

Figure 10. The durations of the lag phase determined by the new method and the traditional method are correlated linearly, which makes it possible to calculate the "new" lag phase duration without complicated mathematical manipulations. Dotted lines represent the upper and lower prediction limits at a 95% confidence level.

Table 1. Correlation between the lag phase duration and the durations of first transitional, exponential, and second transitional phases

Phase	Intercept, a	Slope, b	R^2
First transitional	−0·169	0·822	0·981
Exponential	−0·412	2·011	
Second transitional	−0·448	2·183	

independent of temperature,

$$t_2 = 2{\cdot}454 \times t_1, \qquad t_3 = 2{\cdot}655 \times t_1. \quad \text{Eqn (14)}$$

Furthermore, the durations of exponential and transitional phases can be expressed as a linear function of the lag duration (Eqn (15)), and the parameters for each function are listed in Table 1.

$$t_i = a + b \times t_0, \quad i = 1, 2, 3. \quad \text{Eqn (15)}$$

Although the traditional three-segment linear model was not accurate enough to describe a bacterial growth curve, the lag phase (L) calculated from this model could be used to determine the lag phase (t_0) of a growth curve described by a five-segment multiple linear model with $k_{Tr} = k/3$ (Fig. 10). The lag phase (t_0) of the new multiple linear models was a linear function of the traditional linear model (Eqn (16)). As a result, the parameters (L and k) of the traditional linear model can be used to develop new parameters of a five-segment multiple linear model. With the lag phase (t_0) determined from Eqn (16), the duration of other growing phases (first transitional, exponential, and second transitional) can be calculated from Eqn (15). Consequently, a more accurate five-segment linear growth curve can be generated without numerically solving Eqn (6).

$$t_0 = -0{\cdot}0720 + 0{\cdot}705 \times L. \quad \text{Eqn (16)}$$

Conclusions

This study successfully developed a mathematical methodology to generate multiple linear models that were more accurate than the traditional linear model in describing and categorizing a sigmoidal growth curve for microorganisms. The new multiple linear models were as statistically accurate as the Gompertz model, but progressively divided and categorized the growth curve into five adjacent segments, including lag, first transitional, exponential, second transitional, and stationary phases.

The incorporation of two additional linear models in the transitional regions of a growth curve reduced the error of the traditional linear model dramatically. The selection of the slopes, ranging from 1/5 to 1/2 of the maximum growth rate (k), of the transitional growth phases did not affect the accuracy of the multiple linear models. With the growth rate of the transitional region equal to 1/3 of the maximum growth rate, the duration of each phase, t_0, t_1, t_2, and t_3, was linearly related to each other. This linear relationship among t_0, t_1, t_2, and t_3 was independent of temperature. Such linear relationship would enable the generation an entire growth curve from the maximum growth rate (k) and the duration of any of the growth phase (the lag phase, for example) without involving complicated mathematical and numerical computations. Moreover, the lag phase of the new five-segment multiple linear models was a linear function of the lag phase of the traditional linear model determined from the Gompertz model. With such a relationship, the new more accurate five-segment multiple linear models can be generated from the traditional linear model or the Gompertz equation.

The methodology and mathematical models developed in this paper can be applied directly to the food industry to determine the microbiological shelf-life of foods more accurately. The traditional lag phase and maximum growth rate (or generation time) that are used conventionally in the food industry can be easily converted into parameters of the five-segment multiple linear models which not only predict the growth of micro-organisms accurately, but also indicate clearly its physiological state of growth. As a result, more appropriate intervention technologies can be adopted to control the growth of micro-organisms.

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